Bactericidal activity of 5 kinds of disinfectant on Vibrio cholera isolated from drinking water in Baqupa city.

Khadija Shabban Al-mizury (MSc)[1] and **Amel Mutafa kamil** (MSc)[2]

Abstract

Background: Cholera is an infectious, acute, diarrheal disease caused by bacterium *Vibrio cholerae*. About 3-5 million cases and over 100,000 deaths occur each year around the world. *Vibrio cholera* found in water or food sources that have been contaminated by feces from a person infected with cholera. Cholera is most likely to be found and spread in places with inadequate water treatment, poor sanitation, and inadequate hygiene. Cholera bacterium may also live in the environment in brackish rivers and coastal waters.

Objective: To detect Vibrio cholerae in sources of drinking water in Baquba city and comparing the proportion of contamination with cholera in the two main sources of drinking water namely Tanker and tap water. As well as comparing the efficiency of five types of disinfectants in killing Vibrio cholerae

Materials and Methods: This study was conducted in period from January 2015 to November 2015. Five types of disinfectants in common use have been tested in the laboratory by microtiter Plate. Where identified the inhibitory concentrations and the time required to kill isolates of *Vibrio cholera*. The results showed different inhibitory concentrations for the five types of disinfectants and the time of the killings as follows: Chlorhexedin scored the lesser interval 15 minutes in 128 mg/ l concentration while iodine and Sodium hypochlorite recorded the same and longest killing interval 30 minutes in1500mg/l and 256mg/l concentration respectively, The killing time of ethyl alcohol was 20 minutes at 70% concentration and the killing time of chloroxelenole concentration of 512 mg/l was 25 minute.

Results: Nine isolates were diagnosed of *Vibrio cholera* of the total 174 water samples collected from two sources of water tap water and tanker water equipped mainly for drinking water in the well-known places in the city of Baqupa, 4% of these isolates were found in the tanker water and the rest 1.9% isolated from tap water. The main sources of drinking water in Baquba city were contaminated with *Vibrio cholera* except Baqupa center and Tahrer, while Buhrez occupied the highest contamination proportion .Tankers water occupied the highest proportion of contamination.

Conclusion: The effectiveness of chemical disinfectant against Cholera varies depending on their site of action and concentration. The most effective were chlorohexidine, ethanole, chloroxelenol, respectively. While the lesser effect were sodium hypochlorite and iodine respectively.

Key words: Cholera, chlorhexedin, ethanol, sodium hypochlorite, iodine, water .

Corresponding Author: alaamriziad@gmail.com

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[1.2]Department of Community Health.- College of Health and Medical Technology - Middle Technical University- Baghdad - Iraq.

Introduction

World Health Organization (WHO), 2004 state the largest risk associated with drinking water is the microbial parlous as a result of contamination of water by human and/ or animal feces. All world organization, medical institutes and public health departments realize this danger [1].The problem of billion peoples in the world suffer from lack of access the safe water and at least 2.4 billion of world people suffer from inadequate sterilization processes of water [2].

One point seven million death cases in the worldwide yearly because of poor water quality and sterilization, particularly owing to diarrhea. In developing countries diarrheal disease which transmitted by water affects children under five years. Annual global count mortality from diarrhea ranged from 2.5 to 3.5 million and further than 80% are shared children under five year old [3].

Cholera is a primary disease of marine fish in salt and brackish water. Disease out breaks often occurs in late summer in shallow near shore waters when water temperatures increase. Vibriosis has been reported in over 50 species of salt and fresh water fish and a major obstacle for marine Salmon culture [4].

Vibrio cholerae is the causative agent of cholera which is an infection of small intestine with range of symptoms varied from mild to severe. The known symptoms is a large amount of watery diarrhea continues for few days, vomiting, and may muscle spasm occur, severe diarrhea lead to dehydration and electrolyte imbalance[5]. *Vibrio cholerae* spread mostly by water and food which contaminated by feces of human containing bacteria. Insufficient sanitation of water make serious risk for transition of disease [6]. *Vibrio cholerae* is a grame negative comma shaped facultative anaerobe. It has flagellum at one cell pole as well as pilli [7].

Good quality drinking water supplies reduces the transmission of viral hepatitis A. cholera and acute watery diarrhea but does not influence the incidence of bacillary dysentery [8].

There is large number of methods to treat water to be suitable for consumption .They included physical and chemical ways to removing microorganisms. Each method has advantage and disadvantage and limitations to get disinfected water. Physical methods include boiling water, heating water to pasteurization temperatures and solar water by using uv radiation. While chemical method of disinfection of drinking water which kills all types of microbes, practiced and promoted at the public level and suitable to use in the household level. Chlorine recognized at the community and household level for drinking water disinfection. Chloramines, ozone, and chlorine dioxide are also frequently used as disinfectants, but essentially at the community level. They are mostly difficulties use and preparation therefore they are limited in the community level [9]. Chlorine is a halogen, highly efficient disinfectant, added to public water to kill waterborne pathogen. Its obtain from NaCl ,its liquefies under pressure but its gas at atmospheric pressure. Its oxidizing agent , has and its products neutral charge make it easy to penetrate the negative cell wall and disturbed the integrity of bacterial wall constituents and react with intracellular proteins and enzymes leading microorganism to die or decrease microbial ability to multiply [10].

Chlorine reaction in water explain in the formula $Cl2 + H2O \rightleftharpoons HOCl + HCl$. But in the alkaline solution only CIO is present. Very small amount of $ClO2^-$,$ClO3^-$, ClO4 are also found .while in acidic solution the major result is Cl2 and HOCl[6].

Patients and Methods

Sampling sites and sources: Samples of drinking water was collected from7 different areas known in Baqupa in a period from January 2015 to November 2015. Included two main sources for the processing of drinking water in these areas, a tap water and tanker water.

Collection of water samples: Collecting water from sources followed WHO, 1984[11]. After wearing sterile cloves, the tap water allowed to flow for 2 min .By gas burner and alcohol the interior of the tape sterilized .Then 500 ml of water samples were collected in sterile bottles.

Sampling from the tanker was performed by immersing the sterile bottle into the tanker water to a depth of about 20 cm with the mouth upwards. All samples were transported directly to the microbiology laboratory.

Culture of *Vibrio cholera.* Fifty ml of water samples was inoculated into 25 ml alkaline peptone water [APW]] were incubated for 6 h and then cultured onto thiosulfate citrate bile salt sucrose [TCBS] agar. The inoculated plates were incubated at 37 C_o for 24 h.. Colonies of V. cholera were streaked on gelatin agar [GA] and incubated at 37 C_o to determine the production of gelatinase and then inoculated into kligler iron agar [KIA] and motility indol urea [MIU] agar media. *Vibrio* isolates were tested for lysine and fermentation of glucose, mannitol, sucrose, mannose, arabinose and inositol growth in 0%, 6.5% and 8% Nacl solutions [12].

Minimum inhibitory concentration of disinfectant [MIC]. To assess the bactericidal action of the 5 various chemical disinfectants. Ten ml dilution of each disinfectant prepared and being placed in a micro titer plate and then inoculated with 10 ml of v. cholera suspension to each tube with help of micropipette except negative control. The micro titer plate was incubated at 37 C_o for 24 h and observed for turbidity as a result of bacterial growth by comparing with +ve and –ve control. The turbidity tube was sub cultured on nutrient agar plate and incubated at 37 C_o for 24 h .Then they were examined for the growth of bacteria.

Determination of killing time: The examination by performed 0.1 ml of *Vibrio cholerae* suspension to the tube with the minimum concentration of each disinfectant and then calculate the numbers of bacterial colony by plate count and incubated in 37 C_o for different time .After which the presence or absences of bacteria growth was recorded.

Results

Water intended for drinking up to citizens after the purification processes of purification plants and filter water through the pipes of the stations to homes and the role of institutions directly or it transported by tankers especial for this purpose to the citizens. Although desalination processes that take place on the water, but the results shown in Table 2 appeared to contain water from both sources at the *V. cholera* in all the areas under study are described in Table 1 except tap water in Baqupa Center and Al-Yarmok. From the total 174 water samples found 9 bacterial cells as shown in Table 2 in percentage 5.1% follows 4 % of which tanker water and 1.9% of tap water as shown in table 3.It can be seen from Table 4, less concentration of disinfectants which kill bacteria at least time 15 minutes is 128mg/l chlorhexedine. Followed by 20 minutes killing time for 70% ethanol and 25 minutes for 512 mg/l Chloroxylenol. But equal the killing time of sodium hypochlorite and iodine in 256 and 1,500 mg/l concentration respectively, to achieve the killing time to 30 minutes.

Statistical analysis

The statistical analysis depends comparison the percentage of contamination

present in both water sources as a total percentage for all region (table 3) As well as comparing the less bacteria killing time in less concentration of each disinfectant.

Table (1): Distribution and sources of the water samples.

Site no.	Location	No. samples	sources
1	Baqupa[center]	32	Tap water
2	Bnisaad	26	Tap water
3	Buhrez	38	Tanker water
4	Hay-Al.mustafa	12	Tanker water
5	Shefta	22	Tanker water
6	Tahrer	30	Tap water
7	Al-Yarmok	14	Tap water
	total	174	

Table (2): Numbers of isolates from174 water samples.

Round no.	No. sample	*V. cholera*
1	32	0
2	26	1
3	38	3
4	12	2
5	22	2
6	30	1
7	14	0
Total	174	9

Table (3): Percentage of *V.cholera* according to the source of water samples.

source	Tap water	Tanker water	Total
No. samples	102 [58.6%]	72 [41.3%]	174
No. isolates	2 [1.9%]	7 [4.0%]	9 [5.1%]

Table (4): Minimum inhibitory concentration [MIC] of disinfectants and the time required to kill *Vibrio cholerae*

No.	Disinfectant	MIC.Mg/l	Killing time [min]
1	Chlorhexidine	128	15
2	Ethyl al alcohol	70%	20
3	Chloroxylenol	512	25
4	Sodium hypo chlorite	256	30
5	Iodine	1500	30

Discussion

From 174 drinking water samples there were 9 isolates of V. cholera as shown in table [2], while WHO [2006] guideline state that water intended for human consumption should contain no microbiological agents that are pathogenic to human [13]. The incidence of bacteria in the drinking water agreement with [14] who proved the risk of waterborne disease due to bacterial contamination of potable water despite of state- of- the- art water treatment technology for disinfect, between fifteen to thirty percentages of community diarrheal is a result of contamination of potable water [15].

Bacterial contamination of potable water supplies may be due to corrosion of brass pipe because of chlorine usage as disinfectant that's lead to increase level of copper and lead in the water and provide environment for bacterial growth Other study has shown that maximum corrosion rates occur at 30°C,which coincides with maximum bacterial growth [16]. Also contamination of systemic water in both rural and urban may come from fecal contamination may enter piped water supply due to disinfectant deficiency or infiltration of contamination water or poor source quality [14], or addition untreated water into distribution system[16].
Increased bacterial count in tanker compared to tab water as shown in table [3] of this study may due to sediment buildup of tanker which can act as a growth medium for bacteria in the water [17]. Which leads to persistence of bacteria in the tanker water. In addition to temperature of water in the tanker and increased storage time, these lead to elevate bacterial growth in the tanker water [18] as well as cleaning frequency of tank appears to effect the bacterial growth in the water [18].

So ever the kind of microorganism cell, there is a jointer streak of events. This can be ideates as interaction of the disinfectant with the cell surface followed by penetration into the cell and action at the target site[s]. Cells differ from each other in their nature and composition of surface that's can change due to change in the environment. Interaction at the cell surface can produce a significant effect on viability, but most antimicrobial agents appear to be active intracellular. The outermost layers of microbial cells can thus have a significant impact on their susceptibility [or insusceptibility] to disinfectants and antiseptics[20]. Chlorhexidne seems shortest killing time of Cholera while sodium hypochlorite and Iodine were recorded longer killing time from disinfectants group. That's because of broad spectrum activity of Chlorhexidine against gram-positive and Gram-negative organisms, facultative anaerobes, aerobes, and yeasts [19]. This activity of chlorhexidine depended on its a cationic charge which attracted negative charge of bacterial cell wall with specific and strong

adsorption to phosphate containing compounds. Chlorhexidine is attracted to the inner cell membrane followed the alteration of cell membrane integrity. Chlorhexidine binds to the phospholipids in the inner membrane and there is leakage of low molecular weight compounds like potassium ions, this causes chemically precipitation of the cytoplasm by formation of phosphate complexes include adenosine triphosphate and nucleic acids [21]. While sodium hypochlorite was recorded longer killing time because of its antimicrobial mechanism based on biosynthetic alteration appears in enzyme inhibition and phospholipid destruction that's emerges in lipid peroxidation [22]. Sodium hypochlorite effects by oxidizing the cell of microorganisms and attacking essential cell components including lipid, protein, and DNA [23]. Results were agreement with results Estrela et all [24].

Because of *V. cholerae* is a facultative anaerobe, Iodine appears same killing time of sodium hypochlorite due to its activity on oxygen requirements of bacteria thus impact on respiratory chain by blocking of electron transportation[25]. The result of this study agree with [26].

Some disinfectants typifies as dehydrating agent causing proteins denaturalization rapidly supervened by sub alternation in bacterial metabolism and bacterial lyses [27].This mode of action explains the killing time [20 minutes] of ethanol alcohol. Result agreement with Cases *et al* [28].

Chloroxlenol is a phenolic compound can inhibit the Adenosine triphosphatease [ATPase] which is an important enzyme that is linked to cytoplasmic membrane and thus can inhibit the process of returning potassium ions into cells in exchange for sodium and hydrogen ions [29]. Results also agree with Yakubu *et al* and Olorode *et al* [30,31].

In conclusion. Tanker water and tap water contaminated with *V. cholera*, a major source for the processing of citizens with drinking water in Baquba, except city center and Al Tahrer. While Buhrez occupies the large proportion of contamination. The proportion of cholera cells in tanker water more than that in tap water.

We conclude that the disinfectants vary in effectiveness depending on their site of action in bacteria and concentration therefore chlorhexedine showed the fastest in the killing bacteria in short time bacteria, followed by ethanol and chloroxylenol but the longest killing time was sodium hypochlorite and iodine.

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